

scRNA-Seq data generation

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 An abbreviated version of this protocol was published in Science in Dec 2021

Pan-cancer single-cell landscape of tumor-infiltrating T cells

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Detailed protocol

The tumors and adjacent non-cancer tissues were cut into small pieces (approximately 1~2 mm³) in the RPMI-1640 medium (Gibco, Cat #26140079) with 10% fetal bovine serum (FBS, Gibco, Cat #21875034), and digested by Tumor Dissociation Kit (Miltenyi Biotec, Cat #130-095-929) using gentleMACS™ Octo Dissociator with Heaters (Miltenyi Biotec, Cat #130-096-427), according to the manufacturer's instructions. The dissociated cells were subsequently filtered by 100 µm SmartStrainer and treated with red blood cell lysis buffer (Miltenyi Biotec, Cat #130-094-183) on ice for 1~2 minutes to lyse red blood cells. After being washed twice with 1×PBS (Gibco) and centrifuged at 400×g for 5 minutes each time, the cell pellets were resuspended in the FACS (Fluorescence Activating Cell Sorter) buffer (PBS supplemented with 2% FBS) at the approximate concentration of 10⁶ cells/mL, and then stained with the antibody against human CD45 (ThermoFisher, Cat #11-0459-42, RRID: AB_10852703) and 7-AAD (ThermoFisher, Cat #00-6993-50). CD45⁺ living cells were sorted by flow cytometry with a BD FACSAria™ III sorter (BD Biosciences, USA) for sequencing. 10,000~20,000 cells were used for the 10x Chromium Single-cell 5' and VDJ library construction (10x Genomics, USA), according to the manufacturer's instructions. Purified libraries were subject to HiSeq X Ten sequencer (Illumina, USA) for 150-bp paired-end sequencing.

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1. Gao, R. , Hu, X. and Zhang, Z. (2022). scRNA-Seq data generation. Bio-protocol Preprint. bio-protocol.org/prep1504.
2. Zheng, L., Qin, S., Si, W., Wang, A., Xing, B., Gao, R., Ren, X., Wang, L., Wu, X., Zhang, J., Wu, N., Zhang, N., Zheng, H., Ouyang, H., Chen, K., Bu, Z., Hu, X., Ji, J. and Zhang, Z.(2021). Pan-cancer single-cell landscape of tumor-infiltrating T cells. Science 374(6574). DOI: [10.1126/science.abe6474](https://doi.org/10.1126/science.abe6474)

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